Preparation of an Antigen-Sensitive Hydrogel Using Antigen−Antibody Bindings

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The stimuli-sensitive hydrogels which exhibit swelling changes in response to environmental changes such as pH,[1−3] temperature,[4−14] and electric field[15−17] are promising as intelligent materials because they can sense environmental changes that induce structural changes. The fascinating properties of such stimuli-sensitive hydrogels suggest that they have many future opportunities as suitable materials for mimicking biomolecules and designing smart systems in the biochemical and biomedical fields. This stimuli sensitivity has been mainly caused by release of bound water with large entropy gain or changes in osmotic pressure caused by the polyelectrolyte. For example, hydrogels containing *N*-isopropylacrylamide (NIPAAm) show the temperature sensitivity due to drastic changes in the affinity of polymer chains for water. In this case, the poly(NIPAAm) chain becomes hydrophobic above its lower critical solution temperature (LCST).[4−11] The development of stimuli-sensitive hydrogels through the control of this type of affinity is limited because the result is sensitivity only to simple environmental factors such as pH and temperature. Therefore, there are a few difficulties in developing a stimuli-sensitive hydrogel that is sensitive to a specific molecule. In developing glucose-sensitive systems, such problems have been solved by the combination of enzymatic reactions involving glucose oxidase and pH-sensitive hydrogels.[18−20] In these cases, however, the hydrogels did not respond to glucose directly; the pH-sensitive hydrogels responded to gluconic acid produced from glucose by the enzymatic reaction of glucose oxidase. A few studies on the preparation of stimuli-sensitive hydrogels that directly respond to a specific molecule have been reported.[21−23] However, there are very few reports on the macromolecule-sensitive hydrogels that exhibit swelling changes in response to macromolecules such as proteins and the other biomolecules.

The swelling behavior of hydrogels is governed by not only the affinity of polymer chains for solvent but also the cross-linking density.[24,25] A few researchers have reported that temperature-sensitive hydrogels can be developed by application of reversible cross-linking formations. Okano et al.[13,14] prepared interpenetrating polymer networks composed of poly(acrylamide (AAm)-*co*-butyl methacrylate) and poly(acrylic acid) (AAc) which showed temperature sensitivity due to reversible complex formation between AAm and AAc. By the application of the complex formation between polysaccharides and lectin at cross-linking points in a hydrogel, a glucose-sensitive hydrogel that directly responds to glucose was prepared.[21] These results suggest that a specific molecule-sensitive hydrogel can be prepared by the application of stimuli-sensitive complex formation at cross-linking points in a hydrogel.

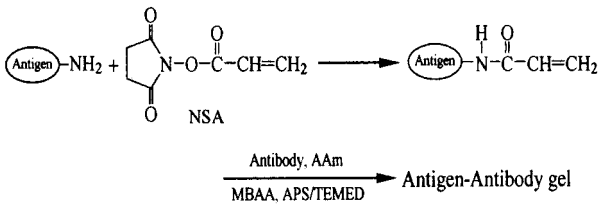

Figure 1. Synthesis of an antigen−antibody hydrogel.

In this paper, a method for preparing a molecule-sensitive hydrogel is reported that involves simple means of introducing stimuli-sensitive cross-linking structures. In this study, we focus on antigen−antibody complex formation because specific antigen recognition of an antibody can provide the basis for fabricating sensing devices with a wide variety of uses for immunoassay and antigen sensing. An antigen-sensitive hydrogel has been prepared by the application of the antigen−antibody binding at cross-linking points in the hydrogel.

The typical method to prepare an antigen−antibody hydrogel is as follows (Figure 1): Rabbit immunoglobulin G (Rabbit IgG) as an antigen was chemically modified by its coupling with *N*-succinimidylacrylate (NSA) in phosphate buffer solution using the method reported by Hoffman et al.[26] NSA (4 mg) was added to a phosphate buffer solution (0.02 M, pH 7.4) containing Rabbit IgG (100 mg), and then the reaction was carried out at 36 °C for an hour to introduce vinyl groups into the Rabbit IgG. After the resulting vinyl-Rabbit IgG was purified by gel filtration, the phosphate buffer solution containing vinyl-Rabbit IgG was adjusted to a concentration of 2 mg/mL. Goat anti-Rabbit IgG (0.74 mg) as an antibody, acrylamide (AAm) (100 mg) and *N*,*N*′-methylenebis(acrylamide) (MBAA) (0.1 wt % relative to AAm) as a cross-linker were dissolved in 900 mg of the phosphate buffer solution containing vinyl-Rabbit IgG (1.48 mg). As soon as 0.02 mL of 0.1 M aqueous ammonium persulfate (APS) solution and 0.02 mL of 0.8 M aqueous *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) solution as redox initiators were added into the mixture, the solution was injected into a glass capillary with an inner diameter of 3 mm, and the polymerization was carried out at 25 °C for 3 h. After polymerization, the hydrogels which were taken out of the capillary and cut into short rods were immersed in a phosphate buffer solution to remove residual chemicals and unreacted monomers. Poly(acrylamide) (PAAm) hydrogel as a reference was also prepared by the redox copolymerization of AAm and MBAA in the same way. The swelling ratio of the resulting hydrogels (V/V_0) was determined from the ratio of their diameters which were measured in the phosphate buffer solution with and without free antigen, $(d/d_0)^3$, using an optical microscope.

After the swelling of the PAAm and antigen−antibody hydrogel attained equilibrium in the phosphate buffer solution, Rabbit IgG as a free antigen was added into the solution. The swelling ratio of the PAAm hydrogel containing no antigen−antibody complex decreased slightly by the addition of a free Rabbit IgG due to

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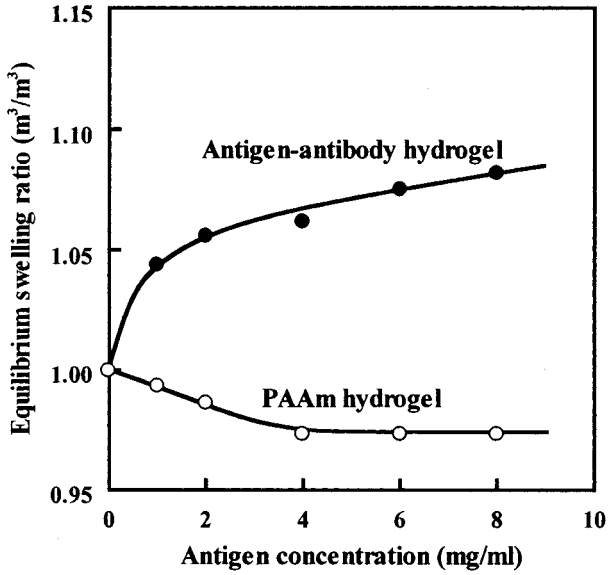

Figure 2. Effect of the antigen concentration in the phosphate buffer solution on equilibrium swelling ratio of the PAAm hydrogel (○) and antigen−antibody hydrogel (●) after swelling attained equilibrium in the phosphate buffer solution containing Rabbit IgG.

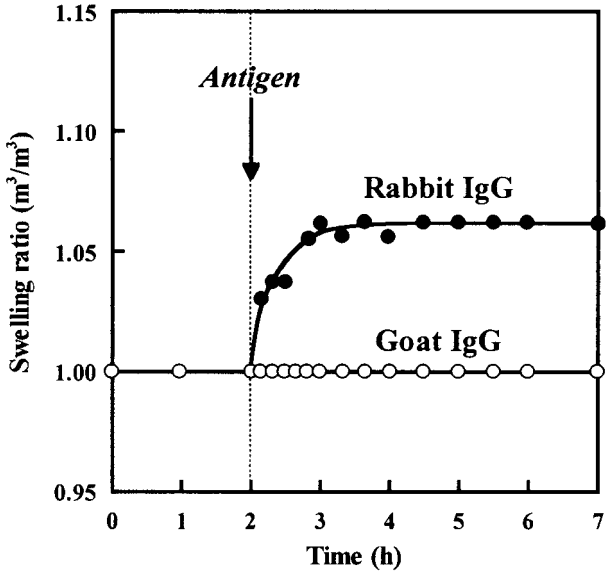

Figure 3. Changes in the swelling ratio of the antigen−antibody hydrogel by the addition of Goat IgG (○) and Rabbit IgG (●) after swelling attained equilibrium in the phosphate buffer solution. The concentration of the antigen in the phosphate buffer solution is 4 mg/mL.

increasing osmotic pressure on the outside of the hydrogel. On the contrary, the antigen−antibody hydrogel was swollen by the addition of free Rabbit IgG. As shown in Figure 2, the swelling ratio of the antigen−antibody hydrogel is strongly dependent upon the free Rabbit IgG concentration in the phosphate buffer solution. This means that the antigen−antibody hydrogel is Rabbit IgG-sensitive.

In general, an antibody forms a complex with a specific antigen due to its strong molecular recognition. Therefore, the swelling changes to the antigen−antibody hydrogel caused by the presence of IgG from a different species were investigated in order to determine whether the effect is due to specific molecular recognition. Figure 3 shows the swelling changes to the antigen−antibody hydrogel caused by the addition of free Rabbit IgG and Goat IgG as a function of time. The addition of free Rabbit IgG resulted in drastic changes in the swelling behavior of the antigen−antibody hydrogel, but the addition of free Goat IgG had no effect. This result suggests that the antigen−antibody hydrogel can recognize only Rabbit IgG and exhibit structural changes. Molecule-sensitive hydrogels reported previously respond to only low molecular weight compounds such as glucose.[18−23] Our findings suggest a simple solution to the problem which is of relevance to many aspects of the development of specific macromolecule-sensitive hydrogels that can respond to macromolecules such as protein and antigen.

The interaction between the modified antigen and antibody was investigated using the BIAcore system[27,28] in which the affinity constant can be determined using surface plasmon resonance. The affinity constants of Goat anti-Rabbit IgG with native Rabbit IgG, vinyl-Rabbit IgG, and its non-cross-linked polymerized Rabbit IgG were 7.04×10^8, 3.27×10^8, and 0.051×10^8 M^{-1}, respectively. The lower affinity constant obtained for polymerized Rabbit IgG as compared to that for free Rabbit IgG is attributable to the denaturation or steric hindrance of the Rabbit IgG by the modification. These results lead to the conclusion that the complex between

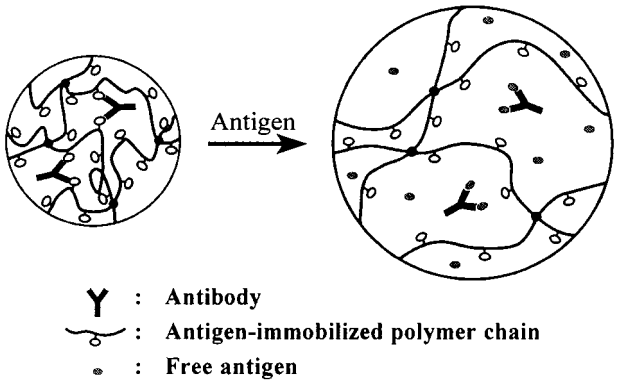

Figure 4. Proposed mechanism for swelling changes to an antigen−antibody hydrogel in response to a free antigen.

Goat anti-Rabbit IgG and Rabbit IgG immobilized in the antigen−antibody hydrogel is dissociated by the addition of free native Rabbit IgG on the basis of the complex exchange between the polymerized antigen and free antigen. The cross-linking density of the antigen−antibody hydrogel was determined by measurements of its compressive modulus.[12,21,24] The cross-linking densities of the antigen−antibody hydrogel were 6 and 4 mol/m^3 in the phosphate buffer solution without and with 4 mg/mL Rabbit IgG, respectively. Furthermore, the cross-linking density of the antigen−antibody hydrogel decreased gradually with increasing free Rabbit IgG concentrations in the phosphate buffer solution. Therefore, the Rabbit IgG-sensitive swelling of the antigen−antibody hydrogel can be explained by the decrease in the cross-linking density in the hydrogel because of the dissociation of the antigen−antibody binding in the presence of a free antigen (Figure 4). As the presence of Goat IgG does not result in the dissociation of the complex between the polymerized Rabbit IgG and Goat anti-Rabbit IgG due to the antigen recognition of the Goat anti-Rabbit IgG, the swelling ratio of the antigen−antibody hydrogel does not change by the addition of Goat IgG.

On the basis of the concepts developed in this study, a variety of specific molecule-sensitive hydrogels can easily be developed by targeting the specific binding or complex formation at cross-linking points in the hydrogels. Such specific molecule-sensitive hydrogels are fascinating materials for the fabrication of intelligent materials and smart systems in the biochemical and biomedical fields, such as biosensors for small molecule analytes, proteins, pathogenic toxins, etc. Now we are trying to prepare another antigen−antibody hydrogel by the immobilization of both antigen and antibody to improve reversible antigen sensitivity. Even though more research into the possible applications for stimuli-sensitive hydrogels prepared using specific complex formation is required, these materials are likely to become quite important in the future.

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